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Review

# Mast cell transcription factors—Regulators of cell fate and phenotype ☆

Sagi Tshori [a], Hovav Nechushtan [b,*]

[a] Department of Medical Biophysics and Nuclear Medicine, Hadassah-Hebrew University Medical Center, P.O. Box 12000, Jerusalem, 91120, Israel
[b] Department of Oncology, Hadassah-Hebrew University Medical Center, P.O. Box 12000, Jerusalem, 91120, Israel

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ABSTRACT

Transcription factors have a key role in mast cell differentiation and response of differentiated mast cells to external stimuli. During differentiation of progenitor cells to mast cells, a role for different GATA transcription factors in combination with PU.1 expression and downregulation of C/EBPα has been described. Notch pathway has been proposed to have a role in mast cell development. The microphthalmia-associated transcription factor expression is upregulated in later stages of mast cells differentiation, but it is not expressed in the closely related basophiles. In differentiated mast cells, there is a role for transcription factors both in determining the specific mast cell phenotype and in the response to immune stimuli such as IgE-Ag. A large number of transcription factors, including AP-1 family proteins, microphthalmia-associated transcription factor and STAT5, are modulated by these stimuli. These transcription factors and related protein modulators form a complex transcription factor network. They can form stimuli regulated specific heterodimers and common inhibitors can move from one protein to another. Transcription factors are the key regulators of mast cell physiology. Modulation of key transcription by such means as the therapeutic siRNA may hopefully allow us to modulate mast cell function, obtaining clinical benefit in a variety of diseases. This article is part of a Special Issue entitled: Mast cells in inflammation.



## 1. Introduction

Mast cells, discovered by Paul Ehrlich more than 130 years ago [1], are evolutionarily ancient cells. Cells containing tryptase–heparin complexes in their secretory granules have been identified in urochordates, which first evolved approximately 500 million years ago. As urochordates lack B and T cells, mast cells presumably predate the appearance of the adaptive immune system [2]. Mast cells are remarkably versatile and are able to secrete a wide range of molecules, such as prostaglandins, chemokines and cytokines [3]. This review focuses on the role of several key transcription factors in mast cells.

While many molecules are involved in cellular regulation, transcription factors are of special interest due to their ability to directly control gene expression. Indeed, some transcription factors may act as master genes, being able to totally change the phenotype of specific cell types. An outstanding example is the ability to obtain dedifferentiated embryonal stem cells from differentiated fibroblasts by the expression of just four transcription factors [4]. Transcription factors also have a critical role in the regulation of most of the cellular responses to external stimuli. In mast cells, the most physiologically important stimulus is the immunological stimulation by IgE and antigen (IgE-Ag). Response to this stimulation is partly regulated by specific transcription factors, as described below. The response is regulated at the level of the immediate induction of the activity specific transcription factors, such as rapid induction of AP-1 activity following IgE-Ag stimulation, as well as in the regulation of the expression of specific proteins needed for immediate response, e.g., the regulation by c-Fos of certain granule proteins needed for mast cell degranulation [5].

Research on transcription factors was always thought as a basic endeavor; however, the potential of gene manipulation by therapeutic siRNA might change this perception [6]. Serious start-up companies and many of the major pharmacologic companies are investing heavily in this therapeutic approach and cooperate with research groups in an effort to identify key targets. Transcription factors are critical regulators and may constitute some of the prime targets for therapeutics. For example, decreasing the expression of the transcription factor C/EBPα alone is sufficient to prevent the formation of both basophiles and committed mast cells [7]. Thus, understanding the intricacies of mast cell transcriptional regulation will hopefully soon have tangible clinical benefits.

## 2. Transcription factors regulating mast cell differentiation

Mast cells are derived from bone marrow precursors. Unlike other bone marrow derived cells, mature mast cells are not present in the blood. Mast cells are confined and are found exclusively in tissues

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* Corresponding author. Tel.: +972 50 8946057; fax: +972 2 6778583.
*E-mail address:* Hovavnech@hadassah.org.il (H. Nechushtan).

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where the final stages of their differentiation take place. The activity of several transcription factors is essential for their initial differentiation; yet, other transcription factors are critically important only during later stage of mast cell maturation.

The understanding of mast cell differentiation is derived mainly from *in vitro* cultures of different stages of stem and progenitor cells. Obviously, these *in vitro* studies are greatly influenced by culture conditions, which may be substantially different from *in vivo* settings. It is not trivial to pinpoint the exact progenitor cell for mast cell development. Some studies claim that mast cell precursors develop from the common myeloid progenitor or the granulocyte–monocyte progenitor cells (CMP or GMP) [7], while some groups have found that, given suitable conditions, several other cell types may differentiate into mast cells [8].

### 2.1. GATA family transcription factors

Manipulations of transcription factors from the GATA family have yielded some impressive results. Initial studies relating to expression of GATA1 and GATA2 revealed expression of GATA1 in both BMMC and mature mast cells. GATA1 knockouts die in the uterus and therefore further studies of the function of GATA1 were performed in mice with lower expression of this gene. Initial studies with heterozygous knockouts for GATA1, which survive to adulthood but have lower expression of this gene, demonstrated in the skin much lower number of Berberine sulfate positive mast cells compared to wild type. This observation provided a strong hint that GATA1 is needed for full maturation of dermal mast cells [9].

Migliaccio et al. [10] and Ghinassi et al. [11] used lower expression of GATA 1 due to lack of the first enhancer and distal promoter of GATA. They revealed two basic defects in mast cells in these kinds of mice. Their first observation is that in these kinds of mice there is a unique trilineage progenitor cell lines committed to erythroid megakaryocytic and mast cell types. Thus, GATA1 has a special role in the development of mast cell progenitor cells. Furthermore, they described in this mice morphologically abnormal Alcian blue positive mast cells and apoptotic mast cell precursors in connective tissue and peritoneal lavage. These results imply that GATA1 has an important role in the final maturation of mast cells and that probably without this transcription factor a large number of mast cell precursors undergo apoptosis. Other studies that added to our information regarding GATA1 role in mast cell physiology was done by Metcalf et al. [12]. Working with bone marrow cells from chimeric mice with inactivated GATA1, they noted the presence of numerous colonies of large cells which turned out to be mast cell progenitors. The majority of the excessive numbers of mast cell progenitors in chimeric GATA1 (Plt13/+) mice were transcribing the inactive Plt13 allele of GATA1, suggesting that GATA1 normally acts to restrict the emergence of committed mast cell progenitors. Interestingly, Metcalf et al. did not detect excessive number of mast cells in these mice, indicating that the progenitor cells probably gave rise to defective mature mast cells. These results are thus similar in their basic conclusion as to GATA1 role in mast cell physiology to those described by Ghinassi et al. although they were obtained in a different system.

Other researchers focused on GATA2. Forced expression of the transcription factor GATA2, together with the transcription factor PU.1, leads to differentiation of mast cells from bone marrow derived myeloid progenitors. However, downregulation of GATA2 in these cells leads to formation of macrophages [13]. Differentiation of mast cells from fetal thymocytes at the double-negative 1 (DN1) and DN2 stages by GATA3 overexpression was achieved by Taghon et al. These mast cells were obtained through manipulations of double-negative T cells at stages 1 or 2 by overexpression of GATA3 in the absence of Notch signaling. Perhaps, more physiologically relevant was their finding that DN2 thymocytes could be induced to differentiate into mast cells by blocking Notch signaling in the presence of the cytokines c-Kit ligand and IL-3, demonstrating that early stage thymocytes are related to mast cells. Is this finding physiologically relevant? This is not clear at all, and to the best of our knowledge, there have not been any experiments demonstrating differentiation of lymphocyte progenitors or early thymocytes into mast cells under any physiological circumstances.

### 2.2. CMP related transcription factors

A recent review has summarized the role of different transcription factors in mast cell development. There seems to be no single "master gene" for mast cell development. Indeed, it seems that a combination of the "correct" expression levels of three transcription factors is essential for the activation of the mast cell differentiation program. The key factors in mast cell differentiation are PU.1 and GATA2/3 since it is not yet clear whether it is GATA3 or GATA2 and the downregulation of C/EBPα (Fig. 1) [14].

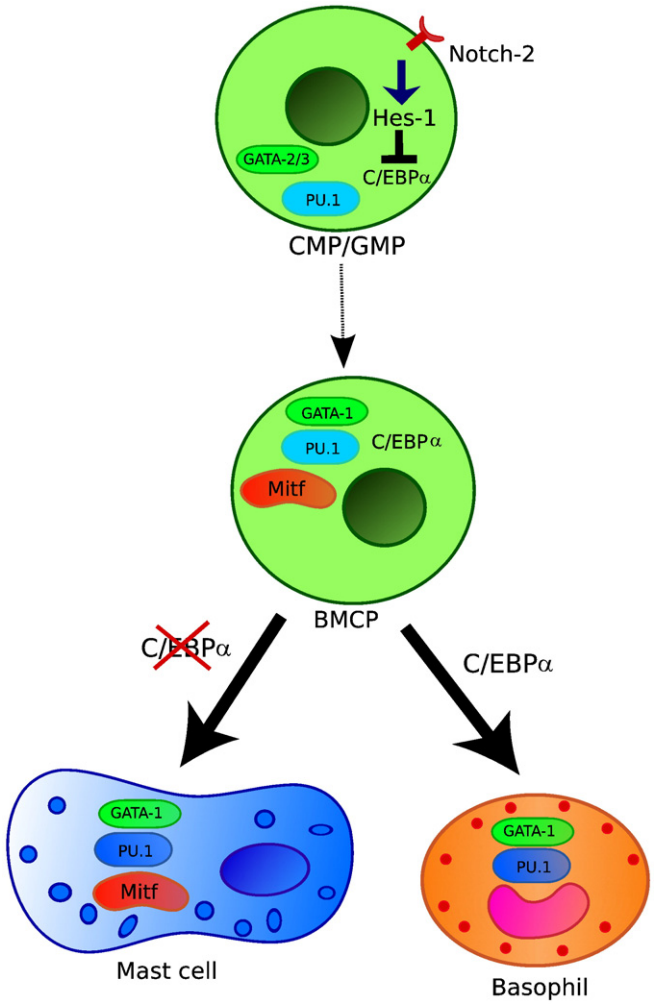


**Fig. 1.** Transcription factors involved in mast cell differentiation. Different experimental systems have yielded slightly different results. It seems that mast cells can differentiate from myelocytes progenitor cells (CMP or from CGP), depending upon the correct level of expression of a GATA factor (GATA2 or 3 depending on the system analyzed) with expression of PU.1 and following downregulation of C/EBPα which can be a result of Hes activation by Notch2. The exact relationship of these cells with the proposed basophile/mast progenitors (BMCP) isolated from the spleen is not clear. However, in these cells, downregulation of C/EBPα with expression of MITF is typical for mast cells and indeed inappropriate expression of C/EBPα will prevent accumulation of mast cells. GATA1 seems to be expressed in these cells and in mast cells; however, it is interesting to note that underexpression of this factor causes shifting of bone marrow progenitor cells towards the production of immature probably non functional mast cells; thus, all these factors should be expressed in a timely fashion in order to get mature mast cells.

## 3. Differentiation from common progenitors of mast cells and basophiles

In an effort to isolate and understand the differences between mast cells and basophiles, Arinobu et al. [15] have isolated a population of cells which are $Lin^-$, c-$Kit^+$ and $Fc\gamma RII/III^{hi}\beta7^{hi}$ from mouse spleen. These cells could differentiate only into mast cells or basophiles. The cells contained Fcε RI mRNA but did not express it on their membrane. This specific cell population, which they named basophile/mast cell progenitors (BMCPs), was expanded in response to allergic stimuli or helminth infection. This expansion in response to known physiological stimulators of mast cell function provides further evidence that BMCPs have a role *in vivo*. These cells were intravenously injected into $W/W^v$ mice, which lack endogenous mast cells and differentiated into mature mast cells detected in the spleen and stomach. Such localization indicated that BMCPs can provide both connective tissue and mucosal mast cells. Analysis of transcription factors revealed that both GATA1 and PU.1 were expressed in basophiles and mast cells as well as in BMCP. However, the microphthalmia-associated transcription factor (MITF), which is essential for mast cell development (see below), was highly expressed in BMCPs as well as in mature mast cells but was absent in basophiles. In contrast, C/EBPα was expressed in BMCPs at a low level and was upregulated in basophiles, while it was significantly downregulated in mast cells. The critical role of C/EBPα in determining the cell fate of these cell populations was demonstrated by overexpression of this transcription factor in mast cells, which led to reprogramming into basophile lineage. However, knockdown of C/EBPα in BMCPs cells induced the exclusive development of mast cell lineage and prevented the development of basophiles. In conclusion, the expression of C/EBPα seems to play a major role in determining the fate of BMCP cells.

### 3.1. Notch as a regulator of mast cell differentiation

The studies above do not address the question of which external cues are essential for the "correct" expression pattern of mast cell differentiation related transcription factors. The recent study by Sakata-Yanagimoto et al. [16] adds much to our knowledge. Sakata-Yanagimoto et al. studied the effects of the Notch receptor mediated signaling pathway on mast cells, which is involved in the differentiation of many cell types, most notably in differentiation and commitment of T cells and marginal zone B cells. Previously, it was noted that mast cells express high levels of Notch2. Sakata-Yanagimoto et al used Delta1-Fc chimeric protein—Delta 1 is one of the ligands of Notch. They studied myeloid progenitors (CMPs) and granulocyte–macrophage progenitors (GMPs) *in vitro*. Sorted CMPs or GMPs of wild type mice were cultured with plate-fixed Delta1-Fc chimeric protein in the presence of c-Kit ligand and IL-3, which yielded cultures highly enriched for mast cells. In the control cultures containing similar mixture of cytokines but in the presence of control antibody instead of a Notch ligand, the main population proved to be granulocytes and macrophages. Under these conditions, activation of the Notch pathway seems to favor the production of mast cells. This is important since the common myeloid precursors are usually regarded as the main source of mast cell precursors [14]. Hairy and enhancer of split 1 (Hes-1) is a Notch responsive basic helix-loop-helix Groucho binding transcription repressor [17]. It has been shown to mediate Notch signaling under a variety of circumstances. Sakata-Yanagimoto et al. analyzed gene expression of CMP and GMP 8 hours following Notch activation noting substantial induction of Hes-1 mRNA in these cells. Interestingly, forced expression of Hes-1 in CMP and GMP cells led to decreased expression of C/EBPα. There was a reproducible increase in the percentage of c-Kit+–$Fc\varepsilon RI^-$ progenitor cells overexpressing Hes-1 but not of cells expressing both c-Kit and FcεRI. Thus, the former population of cells may represent immature precursor cells.

While overexpression of Hes-1 led to the downregulation of C/EBPα, it did not recapitulate the full effect of Notch signaling on mast cell precursors. When Sakata-Yanagimoto et al. analyzed GATA expression levels in the Notch pathway stimulated cells, they noted a substantial increase in GATA3 but not in GATA2 which was previously proposed as the critical factor in mast cell differentiation. Forced expression of both Hes-1 and GATA3 together, but not alone, led to the formation of large colonies of which 80% were mast cells.

It seems that given the correct cytokine milieu, activation of Notch signaling can induce a substantial increase in mast cell. Sakata-Yanagimoto et al. describe unpublished studies of Notch2 knockout mice indicating that these mice have normal mast cell numbers but lower resistance to nematodes [18]. A possible interpretation is that there may be a redundancy between different Notch receptors which can replace Notch2 under most circumstances but not under greater physiological stress, such as during helminth infection. Another possibility is that Notch signaling is not essential for steady state mast cell differentiation.

Summing up the studies described support the view that mast cells are derived from bone marrow progenitors as a result of a shift in the expression profile of a few key transcription factors, which include PU.1, GATA proteins, and C/EBPα. The exact environmental cues that lead to the described transcription factor profile are not clear yet. Cytokines such as c-Kit ligand and IL-3 have a key role but so do different Notch ligands and perhaps other yet unidentified factors.

## 4. Transcription factors in differentiated mast cells

### 4.1. Mast cells subclasses

Like most other immune system cells, there are substantial differences between different mast cells. Initially, it was proposed to divide mast cells to mucosal subtype and connective tissue subtype. With further research, it seemed that mast cell phenotypes are more complex. In the classical experiment, Nakano et al. [19] implanted immature mast cells into mice and demonstrated that their phenotype, assessed mainly by analysis of proteases profile, is determined by their environment and is not predetermined. We are aware of only one effort to systematically search for genes specific for mast cell subtypes. In experiments performed by Tsuchiya et al. [20], mast cells were isolated from the submucosa (sMCs) and mucosa (mMCs) of mouse stomach sections. RNA was extracted, amplified and subjected to microarray analysis. Known marker genes, specific for mMCs and sMCs showed expected expression trends indicating accuracy of the analysis. A total of 1272 genes had significantly different expression levels between sMCs and mMCs. The expression of only one transcription factor, c-Fos, was significantly higher in the sMCs. Their results are interesting because of the proven role of this transcription factor in mast cells. However, the exact function of c-Fos and other transcription factors in determining tissue specific phenotypes still need to be explored. Research on T cells revealed several T cell subtypes capable of producing a discreet subset of cytokines in response to TCR stimulation; yet, no such mast cell subsets have been observed, despite the variety in mast cell phenotypes which is determined mainly by local environmental cues and the fact that in response to similar stimuli mast cells can secrete different immune mediators in different environment. Most researches in mast cells are performed *in vitro*, which may cause such subsets to be missed.

Mast cells have been noted to exert opposite effects on tumor growth [21]. Manipulation of mast cell gene expression will hopefully induce mast cells with clear anti-tumor effects. Such efforts of have been recently reported for neutrophiles creating neutrophiles with anti-tumor effects [22].

## 5. Transcription factors role in signal transduction in mast cells

Transcription factors are key regulators of mast cell transcriptional profile and understanding their role in the specific regulation of different effector molecules secreted from mast cells is critical for any further efforts of changing mast cell gene expression profile.

The most important stimulus of mast cells is immunological activation by IgE-Ag. Protein kinase C (PKC) has been demonstrated to have a major role in mediating the IgE-Ag stimulus. PKC induction following IgE-Ag stimulation was known to induce the AP-1 transcription factor binding to the TPA response element. Rauscher et al. [23] have demonstrated that AP-1 is composed of a heterodimer containing v-Jun transcription factor and c-Fos. Subsequent studies demonstrated that both Jun and Fos are not single proteins but rather the Jun family, including c-Jun, JunB, JunD and Jun binding protein 2 (JDP2), can homodimerize or heterodimerize with other Jun family members or bind the Fos family proteins, c-Fos, FosB, Fra-1 and Fra-2, to form AP-1 transcription factors.

All AP-1 proteins contain a leucine zipper domain. Several other related transcription factors can also bind Fos and Jun family members. For example, Baumruker et al. [24] and Novotny et al. [25] found that in mast cells, cap 'n' collar basic leucine zipper (CNC-bZIP) proteins, which include NF-E2, Nrf1 and Nrf2, can bind to AP-1 proteins upon induction.

Initial studies of mast cells, carried out 20 years ago, demonstrated that IgE-Ag and c-Kit ligand stimulated mast cells display rapid increase in the mRNA levels of several AP-1 family members [26,27]. While both c-Jun and c-Fos are regulated by PKC, c-Jun is regulated by protein accumulation, whereas c-Fos is regulated at the mRNA level (Fig. 2). A complex *in vitro* system was used to determine the PKC isozyme responsible for c-Fos and c-Jun regulation in mast cells [28]. Rat mast cells were chronically treated with TPA, followed by permeabilazation of the cells with streptolysin O, reconstitution with externally supplied recombinant PKC and induction with IgE-Ag. c-Fos and c-Jun expression levels were analyzed. PKC epsilon and PKC beta emerged as responsible for c-Jun and c-Fos induction in this system.

In an attempt to better understand AP-1 complex formation, we began studies of protein complexes containing c-Fos in activated BMMC. Contrary to our assumption, we found that IgE-Ag induced c-Fos is not bound to c-Jun but to a different protein. At that time, a bHLH–leucine zipper protein which was named Fos interacting protein (FIP) was described [29]. Co-immunoprecipitation assays revealed that IgE-Ag activated c-Fos was bound to FIP and not to c-Jun [30,31]. FIP was later found to be identical to the previously described USF2, a bHLH–leucine zipper transcription factor.

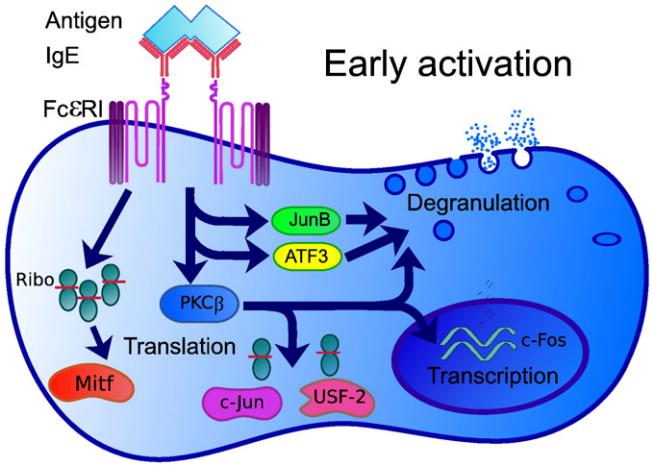


**Fig. 2.** Early events following IgE–antigen stimulation. The schematic cell describes the early signaling events in mast cells within minutes after IgE–antigen stimulation. Antigen interaction with IgE bound to high affinity Fc receptors (FcεRI) activates degranulation through various signaling cascades. Degranulation is dependent upon the activity of PKCβ, JunB and ATF3 since knockout of these genes results in decreased degranulation. c-Fos mRNA is rapidly transcribed after PKCβ activation. USF2, c-Jun and MITF are regulated by the accumulation of protein (seen translated in the ribosomes). c-Jun and USF2 accumulation is mediated through PKCβ.

PKC beta antisense was used to analyze effects on USF2 binding. Downregulation of this protein resulted in decreased binding of USF2 to DNA in IgE-Ag stimulated cells [30]. In another study, general PKC inhibitors were used to study the regulation of USF2. It seemed that USF2 was regulated at the translational level as was demonstrated by the use of polysomal profiling and determined that the regulator of the translation of this protein was PKC [32] (Fig. 2).

Other groups have described the existence of other AP-1 transcription factor complexes. For example, Novotny et al. [25], studying the IgE-Ag regulation of the TNF alpha promoter in mast cells, have described a complex containing Nrf1, a cap 'n' collar transcription factor, with c-Jun, JunD, FosB and ATF2. They also described novel splice variants of Nrf1 in mast cells, which they hypothesize are the reason for the differential binding of Nrf1 to AP-1 family proteins in mast cells as compared to other cells. Baumruker et al. [24,33] also described a specific role for NFAT-1 in TNF alpha regulation in mast cells in addition to the AP-1 complex and in addition to a GATA factor in the regulation of IL-5. NFAT is in fact a description of several important transcription factors. Already in 1995, it has been shown that NFAT is induced following mast cell IgE-Ag stimulation [34]. Furthermore, this transcription factor seems to have an important role both in the production of the cytokine IL-13 [35] and in the prevention of mast cell apoptosis following IgE-Ag stimulation [36]. Interestingly, this is achieved by stimulation of the prosurvival A1 gene which seems to be mast cells specific since in macrophage this is achieved by stimulation of NFk b [36]. Thus, NFAT transcription factors have also an important role in mast cell regulation at least in some cases due to their ability to costimulate gene transcription with GATA transcription factors.

ATF3 is a member of the ATF/CREB (CRE-binding protein) family, which is a subfamily of the AP-1 group. ATF3 has been reported to activate transcription as a heterodimer with c-Jun, whereas it represses transcription as a heterodimer with JunD [37]. It is closely related to the transcription factor Jun dimerization protein 2 (JDP2), which can bind Jun and under certain circumstances downregulate it. JDP2 can act as an inhibitor of ATF3 expression. Most recently through the use of BMMC, it was established that BMMC lacking ATF3 have decreased degranulation in response to IgE-Ag stimulation, whereas IL-4 and IL-6 expression was enhanced [38]. BMMC from ATF3 deficient mice are dependent on both IL-3 and c-Kit ligand induction for their proper differentiation, as opposed to normal BMMC which require only IL-3, probably as a result of downregulation of the AKT pathway [38]. We have earlier demonstrated that degranulation is impaired in PKCβ deficient [39] and in c-Fos deficient mast cells [5]. Similar effects were seen in JunB [40] and ATF3 knockout mast cells [38]. However, there are differences between the cytokine profiles in mast cells derived from each of these knockout mice. Both IL-6 and IL-4 are elevated in ATF3 knockout mice, while only IL-6 is elevated in c-Fos and Jun-B knockout mice. In contrast, substantial reduction in the level of IgE-Ag induced IL-6 expression is demonstrated in PKCβ knockout mice.

These results suggest that a complex network of AP-1 related proteins is involved in the transcriptional control of activated mast cells.

As described above, we noted that USF2 can bind to c-Fos in activated mast cells [31]. USF2 is a transcription factor that is most similar to the bHLH–leucine zipper transcription factors of the MiT family. This family contains the transcription factor MITF [41]. The MITF gene resides at the *mi* locus in mice and mutations of this gene result in deafness, bone loss in dominant negative *mi*/*mi* mice, small eyes and poorly pigmented eyes and skin and in some mutants nearly

total deficit of mast cells [41,42]. As described above, MITF expression was detected in mast cells derived from the common basophile/mast cell committed progenitors (BMCPs) but not in basophiles [15].

MITF contains a basic domain followed by helix-loop-helix (HLH) domain and a leucine zipper domain. The leucine zipper domain is essential for protein–protein interaction and for DNA binding and may allow interaction with other leucine zipper domain containing transcription factors, such as c-Fos and USF2 [43]. Following the identification of the MITF gene, both its function and regulation were studied in mast cells. Kitamura et al. demonstrated in a series of studies (reviewed in [44,45]) that MITF regulates the expression of various mast cell proteases (MCP), including mMCP-6, mMCP-5 (23), the most important mast cell cytokine receptor—c-Kit, and other enzymes such as Granzyme B.

We tried to elucidate the regulatory mechanisms of this transcription factor. In our initial study, we demonstrated that MITF could bind USF2 in IgE-activated mast cells [43]. Furthermore, following this stimulation, its protein levels were increased but not its mRNA levels. MITF has multiple mRNA isoforms due to the use of different promoters and alternative splicing. MITF can be expressed from different promoters (for example, M, A and H). The function and importance of different MITF isoforms is not yet fully understood. Recent evidence using transient transfection of different MITF isoforms suggest that there is differential regulation of some of those forms and also sometimes specific physiological roles [46,47]. For example, only cells overexpressing MITF-e adhere to the culture flask with a morphologic appearance reminiscent of macrophages [46].

We were interested in finding proteins which regulated MITF activity in mast cells. Using yeast two hybrid methodology, we cloned several proteins which could bind MITF with high avidity, including Hint-1, previously known as PKCI [48].Using luciferase assays, we noted that this protein can inhibit MITF transcriptional activity. Furthermore, we noted that Lysyl tRNA synthetase (LysRS), a protein with not only a major role in translation but also a multitude of other "moonlighting" functions [49], binds MITF. LysRS can produce the dinucleotide Ap4A, and we demonstrated that this dinucleotide can release Hint from MITF and allow transcriptional activation [50]. In addition to MITF, Hint could also bind and inhibit USF2 in mast cells [51]. We could also demonstrate that LysRS activity is regulated by phosphorylation through MAP kinase pathway and that transfection of pseudophosphorylated LysRS increased cellular Ap4A levels [52]. Ap4A is degraded by Ap4A hydrolase (NUDT2), and this degradation is induced by IgE-Ag activation of mast cells. Inhibition of Ap4A hydrolase indeed induced MITF target gene expression in mast cells [53] (Fig. 3).

Another inhibitor of MITF that was found by the use of the yeast two hybrid system is the protein inhibitor of stat, PIAS3. It was first described as an inhibitor of STAT3 but serve as an inhibitor of various transcription factors [54]. Interestingly, in IgE-Ag activated mast cells, PIAS3 was released from activated MITF and was bound to STAT3 [55] (Fig. 3). Whether, under physiological conditions, the released PIAS3 can also bind other major transcriptional regulators of mast cells, such as STAT5, is not clear yet. MITF may act not only as a transcription factor but also as a "sink" for PIAS3; thus, regulating the inhibition of various transcription factors.

## 6. STAT5

The JAK–STAT pathway has a critical role in the signal transduction of many cytokines. There are 4 known JAK proteins which can activate one or more of the seven known STAT proteins. We described above the possible interactions between MITF and STAT3. However, there is more data regarding the critical role of STAT5 in mast cells. Several key stimuli of mast cells can induce this transcription factor (there are actually two closely related STAT5 genes and gene products). It seems that the stimulation of this transcription factor is essential for the full IgE-Ag mediated mast cell activation which include degranulation, cytokine production and survival [56]. Furthermore, it seems that in mast cells c-Kit stimulation results in STAT5 stimulation which has a critical role in these cells unlike other cell types where STAT3 is also stimulated by this receptor [57]. Since c-Kit lacking mice are nearly devoid of mast cells, it is not surprising to note that knockout mice of STAT5 almost lack mast cells [58]. BMMC from these mice could only be obtained in the presence of both c-KiT and IL-3 which do seem to stimulate also STAT3 [58].Thus, STAT transcription factors and mainly STAT5 seem to have a critical role in the survival of mast cells and their response to external stimuli.

While we have strived to describe the roles of some of the key transcription factors in mast cells, it is obvious that we have not described all the transcription factors active in these cells and have

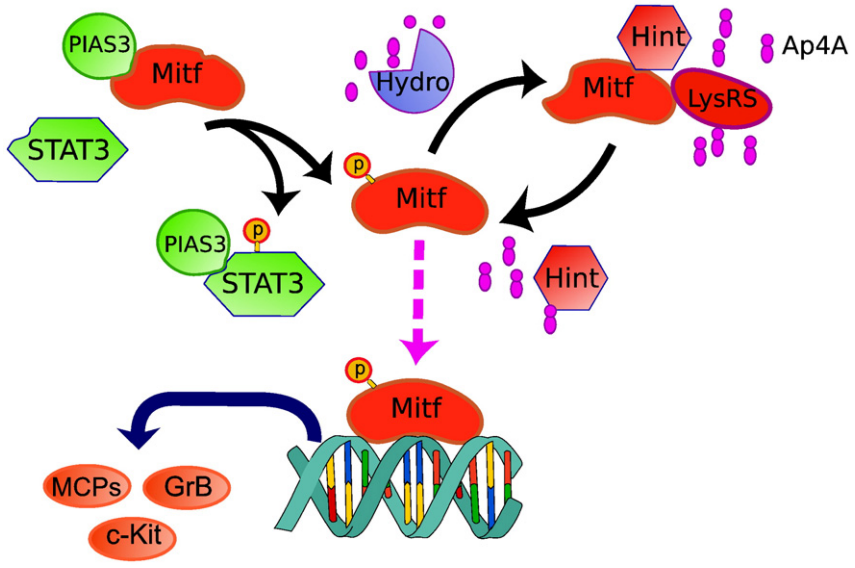


**Fig. 3.** Regulation of MITF transcriptional activity. MITF has been shown to be critical for the regulation of several key mast cell molecules including c-Kit and mast cell proteases (MCPs). In resting mast cells, MITF transcriptional activity is regulated by several repressors, including Hint and PIAS3. During mast cell activation, Ap4A synthesized by MITF bound Lysyl tRNA synthetase (LysRS) and releases MITF from Hint. Ap4A is later degraded by Ap4A hydrolase (Hydro), and Hint can once again bind and repress MITF. MITF is also inhibited by the sumo ligase PIAS3. Upon immunological stimulation, PIAS3 releases MITF and binds the newly phosphorylated STAT3. MITF is now free to transactivate its target genes, such as c-Kit, granzyme B (GrB) and mast cell proteases (MCPs).

omitted some which may also have an important role in these cells such as NFAT [36,59,60] and EGR1 [61–63].

## 7. Conclusion

Transcription factors have a key regulatory role in the differentiation of mast cells, their survival and immune regulated functions. Specific master gene with unique mast cell expression pattern has not been found. Rather than that, it is a combination of several transcription factors which determine mast cell differentiation, survival and function. Differentiation of mast cells seems to be dependent upon the correct expression pattern of a number of key transcription factors. Forced expression of a critical transcription factor can lead to outstanding results for example the transdifferentiation of basophiles into mast cells.

The physiological regulation of these key transcription factors *in vivo* is only now beginning to be understood. It seems that some known receptors such as those from the Notch family play a key role also in the regulation of mast cell progenitors besides their better characterized roles in other cells such as T cells.

In differentiated mast cells, the picture that emerges is even more complicated. Numerous transcription factors are active in mast cells. Their activity is modulated by external stimuli such as IgE-Ag and cytokine receptors. Many of them are found in complexes and can change their binding partners upon external stimulation. The possible number of complexes is very large. New techniques allowing the isolation of chromatin bound proteins, isolation of large protein complexes and their analysis by mass spectrometry will provide new data regarding the active complexes present in mast cells *in vivo.*

Critical for our understanding of the specific roles of transcription factors in mast cells has been the use of knockout mice. Using these models, it has been found out that there are differential roles even for closely related proteins such as those from the AP-1 protein family. Knockout mice were also instrumental for our initial understanding of some key proteins regulating several mast cell factors such as PIAS3 regulating both STAT3 and MITF.

In order to better study *in vivo* mast cell specific activity of transcription factors, it would be important to create conditional knockout mice with mast cell specific expression and also inducible gene regulation. Initial mice with mast cell specific gene ablation have been described and we hope they would be soon useful for transcription factors studies in mast cells.

Investigating the transcriptional networks controlling mast cell function is a difficult task. Yet it is crucial for our in-depth understanding of the mast cell regulation. Hopefully, such studies would provide us with the insights necessary for the formulation of sophisticated therapeutic strategies of these key immune cells.

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